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## SPECIES VARIATION IN NORMAL BLOOD LIPIDS ESTIMATED BY OXIDATIVE MICROMETHODS<sup>1</sup>

BY ELDON M. BOYD<sup>2</sup>

### Abstract

The blood plasma of 10 healthy bullfrogs and of 15 healthy, adult dogs was analysed for lipids by oxidative micromethods. The lipid content of the blood plasma of bullfrogs was found to be low and of the order of that found previously in guinea-pigs with, in addition, the peculiar feature that no neutral fat was present. The lipid content of the blood plasma of the dog, on the other hand, was found to be high, higher even than is found in man. The lipid composition of blood plasma in bullfrogs and dogs was then compared with corresponding values previously determined by the author, using the same technique, in guinea-pigs, albino rats, rabbits, cows, cats, cockerels, and man. Data are also presented upon the concentration of lipids in the red blood cells of bullfrogs and these are compared with corresponding values previously found in rabbits, man, and guinea-pigs.

### Introduction

In his recent excellent monograph on the biochemistry of lipids, Bloor (1) remarks that "the determination of normal values (of blood lipids) is fundamental." He notes further that "The values found are often contradictory and confusing because of the variety of methods used." For these reasons, it is valuable to have available figures for the various blood lipids as determined by the same analyst with the same method. Even the same analyst may obtain different figures for normal animals from time to time. For example, the values for normal human plasma lipids published by Boyd in 1933 (2) differ somewhat from those of a much larger series of individuals analysed and reported by Boyd in 1937 (4).

Two years ago, the author (7) published a report in which were assembled data upon the plasma lipids of a group of normal animals. No attempt has been made to investigate systematically the species variation in normal blood lipids, but as different species of animals have become available in connection with other investigations, their normal blood lipid values have been estimated.

### Material and Methods

Since the report of two years ago, the plasma lipids have been determined in two additional species, the bullfrog, *Rana catesbiana*, and the dog (various breeds, mostly mongrels). In both species, the animals were adult, healthy

<sup>1</sup> Manuscript received January 4, 1944.

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specimens of both sexes. The bullfrogs were used soon after they had been captured from marshes and swamps in the summer months. During the short interval of a few days that they were kept in captivity, they were placed in a large frog pond in the basement of this University building, along with other small frogs, chiefly leopard frogs. When the large bullfrogs were killed, practically every one was found to have a leopard frog, or the disintegrating remains of a leopard frog, in its stomach, so that their diet was, at least in part, carnivorous. The dogs were fed upon Purina Fox Chow Checkers supplemented with skimmed milk and scrap meat. All animals had been without food for 15 hr. or so before blood was withdrawn for analysis. In the dogs, venous blood was used; in the bullfrogs, blood was taken from the heart. The drawn blood was oxalated, which of course dilutes the plasma; this has been the standard practice of the author with other species. Blood plasma, and in bullfrogs the red blood cells, were then extracted and analysed for various lipids by the author's several modifications of the Bang, Bloor, Windaus, etc., methods (5).

### Results and Discussion

Data upon the lipid composition of blood plasma of 10 bullfrogs and 15 dogs were tabulated, means and standard deviations calculated, and these figures added to a table of corresponding values for guinea-pigs, albino rats, rabbits, cows, cats, cockerels, and man as previously reported (7). The collected data are assembled in Table I.

TABLE I  
THE LIPID COMPOSITION OF BLOOD PLASMA OF NORMAL ANIMALS  
AS ESTIMATED BY OXIDATIVE MICROMETHODS

(The results are expressed as mean  $\pm$  standard deviation mgm. per 100 ml. of plasma)

Lipid	Species*								
	Guinea-pig (10)	Bull-frog (10)	Albino rat (116)	Rabbit (89)	Cow (3)	Cat (27)	Cockerel (22)	Man (118)	Dog (15)
Total lipid	169 $\pm$ 34	204 $\pm$ 32	230 $\pm$ 31	243 $\pm$ 89	348 $\pm$ 51	376 $\pm$ 110	520 $\pm$ 85	530 $\pm$ 74	590 $\pm$ 133
Neutral fat	73 $\pm$ 33	0 $\pm$ 0	85 $\pm$ 30	105 $\pm$ 50	105 $\pm$ 39	108 $\pm$ 65	225 $\pm$ 77	142 $\pm$ 60	182 $\pm$ 74
Total fatty acids	116 $\pm$ 29	82 $\pm$ 21	152 $\pm$ 23	169 $\pm$ 66	202 $\pm$ 55	228 $\pm$ 82	361 $\pm$ 74	316 $\pm$ 85	386 $\pm$ 105
Total cholesterol	32 $\pm$ 5	104 $\pm$ 18	52 $\pm$ 12	45 $\pm$ 18	110 $\pm$ 32	93 $\pm$ 24	100 $\pm$ 23	152 $\pm$ 24	110 $\pm$ 28
Ester cholesterol	21 $\pm$ 4	73 $\pm$ 18	31 $\pm$ 10	23 $\pm$ 12	73 $\pm$ 15	63 $\pm$ 23	66 $\pm$ 19	106 $\pm$ 25	59 $\pm$ 19
Free cholesterol	11 $\pm$ 2	31 $\pm$ 3	21 $\pm$ 8	22 $\pm$ 13	37 $\pm$ 15	30 $\pm$ 10	34 $\pm$ 9	46 $\pm$ 8	51 $\pm$ 20
Phospholipid	51 $\pm$ 12	52 $\pm$ 16	83 $\pm$ 24	78 $\pm$ 33	84 $\pm$ 21	132 $\pm$ 53	155 $\pm$ 34	165 $\pm$ 28	242 $\pm$ 80

\* The numbers in parentheses after the name of each animal indicate the number of animals examined.

The total lipid content of the blood plasma of bullfrogs was low but not lower than that of some mammals, such as guinea-pigs. The peculiar feature of bullfrog blood plasma was the complete absence of neutral fat, a finding

which the author has not encountered in the blood plasma of any other species of animal.

Dog plasma was rich in lipids compared with that of other mammals. The mean total lipid of dog blood plasma is the highest yet encountered in any species of animal whose plasma has been analysed by the author.

From the earliest reports of analyses of blood plasma lipids, investigators have compiled tables in which they have shown that while the total amount of the various lipids may vary, the ratios of one or another lipid to each other remain fairly constant. Thus there are many "lipid constants", "lipemic coefficients", "lipemic constants", and various "lipid ratios" and the number of possible combinations is large indeed. All of these may be simplified, and yet the general idea maintained, by adopting a plan proposed by Boyd in 1937 (4). There are essentially four main groups of lipids in blood plasma, namely, neutral fat, cholesterol esters, free cholesterol, and phospholipids. When the percentage of each of these in the total plasma is calculated, it is found that no matter what the total lipid of normal blood plasma may be, its percentage composition is remarkably constant—at least for such birds and mammals as have been examined by the author.

The percentage composition of total lipid of blood plasma of nine species of normal animals has been compiled in Table II. The percentages of neutral fat, free cholesterol, and phospholipid have been calculated directly from the values given in Table I. Cholesterol esters have been estimated as five-thirds the value recorded as "ester cholesterol" for reasons that have already been explained (4).

TABLE II

THE PERCENTAGE COMPOSITION OF TOTAL LIPID IN THE BLOOD PLASMA OF NORMAL ANIMALS

	Neutral fat, %	Cholesterol ester, %	Free cholesterol, %	Phospholipid, %
Guinea-pig	43	21	7	29
Bullfrog	0	60	15	25
Albino rat	37	22	9	36
Rabbit	43	16	9	32
Cow	30	35	11	24
Cat	29	28	8	35
Cockerel	43	21	7	30
Man	27	33	9	31
Dog	31	17	9	41
Mean of mammals and birds	35	24	9	32
Standard deviation	6.5	6.6	1.3	4.8

If the peculiar distribution of plasma lipids in the bullfrog be excluded, then it becomes apparent that the percentage composition of plasma total lipid is remarkably constant from species to species. Thus, though dog plasma has some three times as much lipid as guinea-pig plasma, the percentage distribution of total lipid remains about the same in the two species.

It is quite obvious that the percentage composition of plasma total lipid—and hence the various ratios, constants, and coefficients—is much more constant from species to species (as shown in Table II) than are the lipid values expressed as mgm. per 100 ml. (as shown in Table I).

While the lipid content of plasma varies considerably from species to species, the lipid content of the red blood cells is almost the same in all species studied by the author to date. This was demonstrated many years ago but is not generally appreciated, even to-day. The author has stressed the point many times that plasma or serum should be selected for lipid analysis, rather than whole blood, if only one analysis or one set of analyses is to be done. The lipids of the red blood cells are not entirely insensitive to variation, so that a truly complete study of blood lipids should really include an analysis of both plasma and red blood cells.

Such being the case, it is again essential that the lipid content of normal red blood cells be known. The author has previously reported upon the lipid content of the red blood cells of rabbits (6), man (4), and guinea-pigs (8). These data, all upon the lipid content of red blood cells from oxalated blood, have been augmented by data upon the large, nucleated red blood cells of the bullfrog and the whole assembled in Table III. It is apparent that the total lipid of the red blood cells is fairly constant at about 0.5% in all species, in spite of the wide variation in the concentration of plasma lipids.

TABLE III

THE LIPID COMPOSITION OF THE RED BLOOD CELLS OF NORMAL ANIMALS  
AS ESTIMATED BY OXIDATIVE MICROMETHODS

(The results are expressed as mean  $\pm$  standard deviation mgm. per 100 ml. of red blood cells)

Lipid	Species*			
	Bullfrog (10)	Rabbit (67)	Man (66)	Guinea-pig (10)
Total lipid	448 $\pm$ 47	433 $\pm$ 66	523 $\pm$ 86	517 $\pm$ 60
Neutral fat	0 $\pm$ 0	63 $\pm$ 56	84 $\pm$ 63	47 $\pm$ 28
Total fatty acids	188 $\pm$ 26	251 $\pm$ 57	294 $\pm$ 66	282 $\pm$ 36
Total cholesterol	195 $\pm$ 20	112 $\pm$ 16	123 $\pm$ 36	119 $\pm$ 19
Ester cholesterol	79 $\pm$ 18	5 $\pm$ 9	8 $\pm$ 9	8 $\pm$ 7
Free cholesterol	116 $\pm$ 16	107 $\pm$ 15	115 $\pm$ 27	112 $\pm$ 15
Phospholipid	201 $\pm$ 32	259 $\pm$ 35	311 $\pm$ 57	347 $\pm$ 48

\* The numbers in parentheses after the name of each animal indicate the number of animals examined.

The total lipid of the red blood cells may be viewed as made up of four essential groups of lipids, just as was plasma. In the mammals, total red blood cell lipid was distributed almost identically between these four groups of lipids in each of the species studied. On the average, it was composed of 13% neutral fat, 3% cholesterol esters, 23% free cholesterol, and 62% phospholipids.

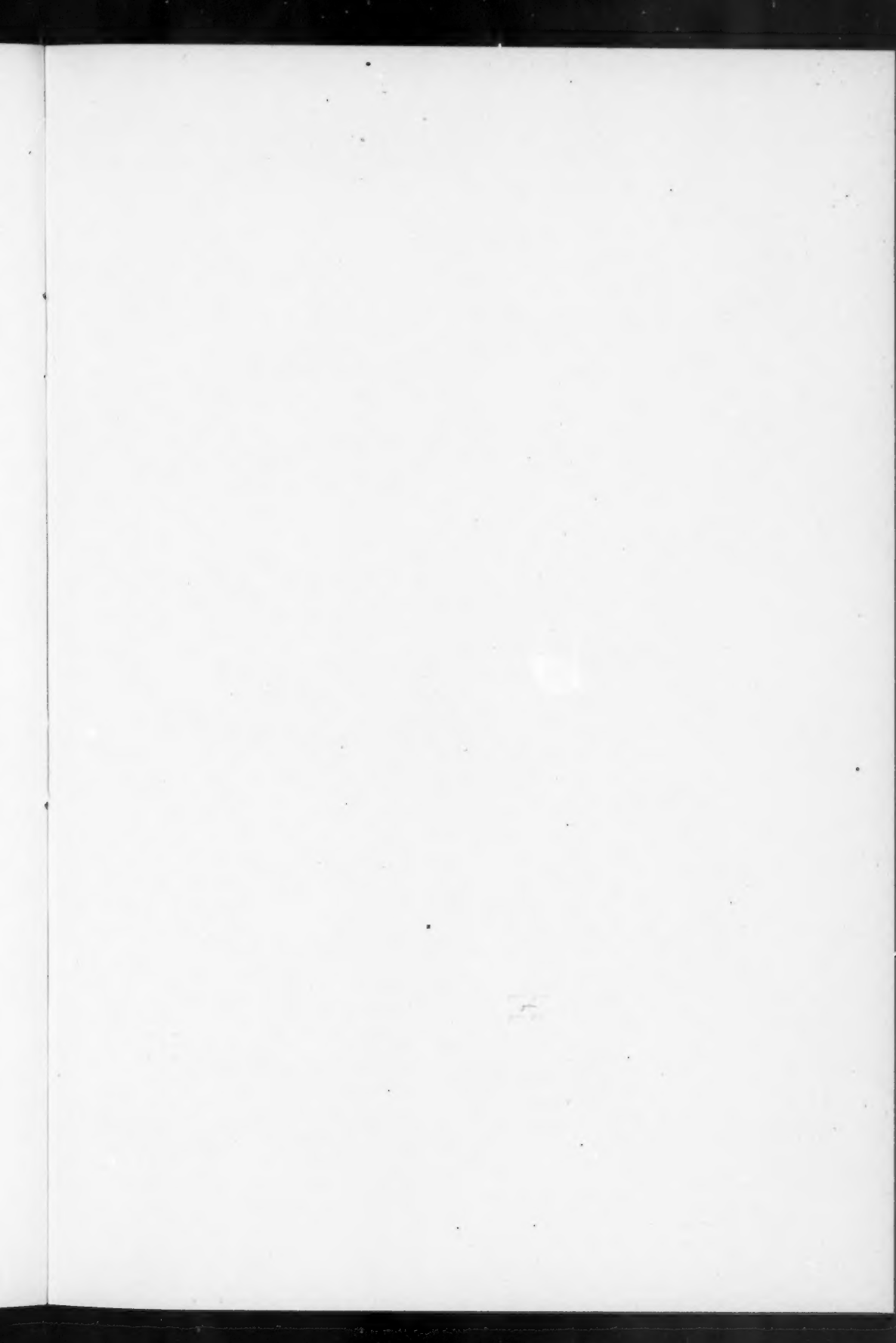
The red blood cells of bullfrogs had no neutral fat, and in addition had a much larger amount of cholesterol esters than had mammalian red blood cells. These were the two chief differences between the lipid content of the red blood cells of mammals versus that of bullfrogs, although the amount of phospholipids was somewhat lower in the bullfrogs.

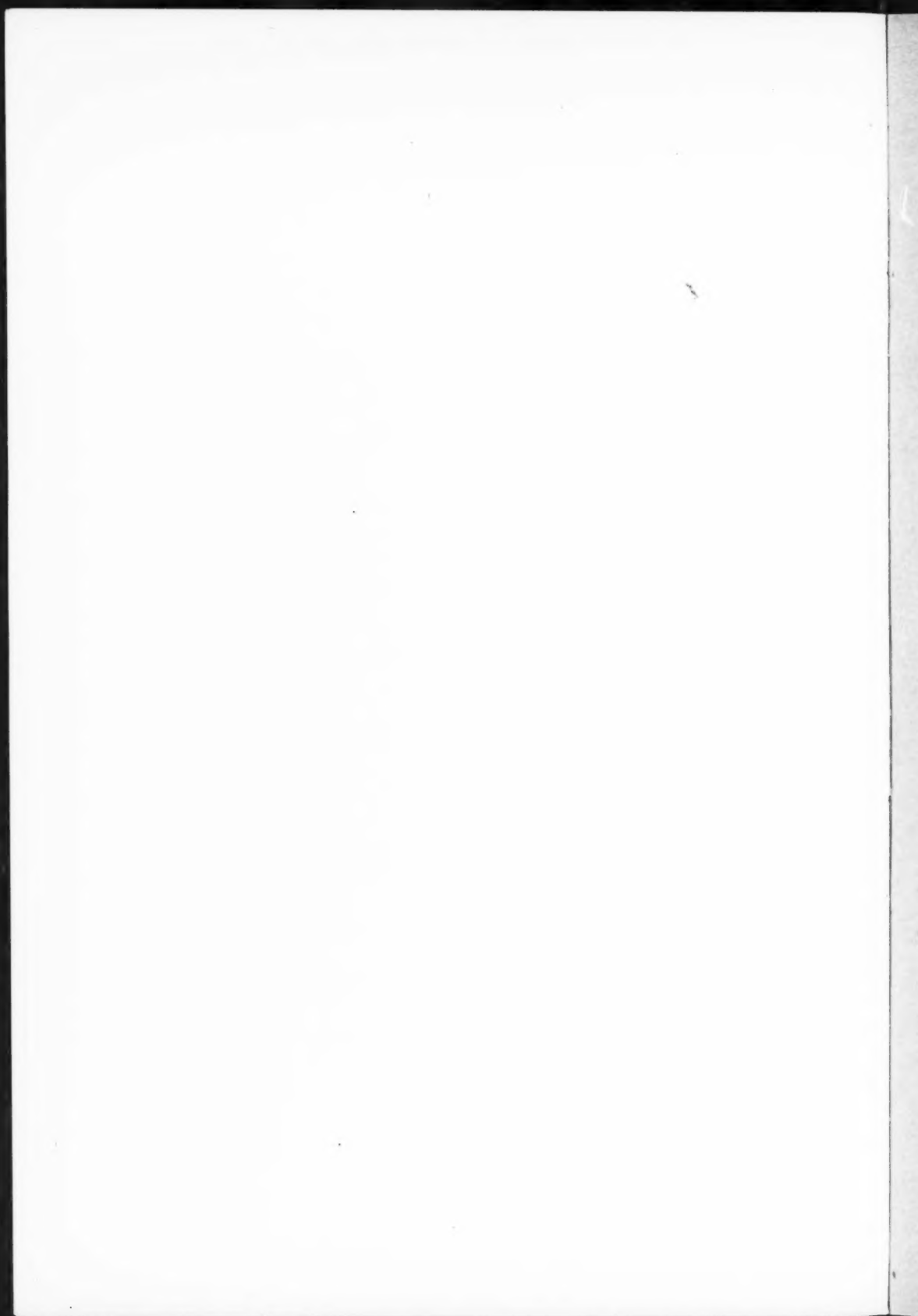
Bloor (1) has likened the lipid content of the red blood cells to that of body tissues. While this is true in one respect, namely, the similarity amongst various species, it is not true with respect to the total amount or distribution of lipids. The total lipid content of most tissues averages at least 2% wet weight and the large portion of it is neutral fat. No broad generalization can be made because of the wide variation in tissue lipids from one organ to another. The tissues of the umbilical cord (Jelly of Wharton), for example, contain only about one-half the lipid of the red blood cells and the percentage composition is more or less similar (3). The red blood cells being non-nucleated, it might be expected that their lipid content would differ from that of ordinary tissues composed of nucleated cells. On the other hand, it was anticipated that the nucleated red blood cells of the bullfrog might have shown a different lipid content from the red blood cells of mammalian blood.

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